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FEATURES OF IMMUNE STATUS IN DIFFERENT STATES OF URIC ACID METABOLISM IN FEMALE RATS

Anatoliy I Gozhenko[1], Vadym S Smagliy[1], Inna V Korda[2], Nataliya S Badiuk[1], Walery Zukow[3], Igor L Popovych[1,4]

[1]Ukrainian Scientific Research Institute of Medicine for Transport, Odesa
prof.gozhenko@gmail.com; badiuk_ns@ukr.net
[2]IY Horbachevs'kyi National Medical University, Ternopil' kordai@tdmu.edu.ua
[3]Nicolaus Copernicus University, Torun, zukow@umk.pl
[4]OO Bohomolets' Institute of Physiology, Kyïv i.popovych@biph.kiev.ua

Abstract

Background. Previously, we found a wide range of uric acid metabolism parameters and functional relationships of uricemia and uricosuria with the parameters of immunity in healthy female rats analyzed. It was found by canonical correlation analysis that both parameters of uric acid metabolism determine the immunity status of rats by 71%. The purpose of this study is to determine the features of immune status in rats with quantitatively and qualitatively different uric acid metabolism. **Material and Methods**. Experiment was performed on 60 healthy female Wistar rats 220-300 g. Among them 10 animals remained intact, using tap water from drinking ad libitum. The rats of others groups for 6 days administered through the tube various fluids. The serum and urine levels of the uric acid (uricase method) were determined. In the blood, the parameters of immunity were determined. From thymus and spleen made smears-imprints for counting splenocytogram and thymocytogram. For them, as well as immunocytogram and leukocytogram of blood, Shannon's entropy was calculated. **Results.** Screening of immune profiles of four quantitative and qualitative uric acid metabolism clusters revealed 6 functional-metabolic patterns, two of which are quasilinear (enhancing and suppressing), with immune extremes at moderately elevated uricemia, two U-shaped with immune optimum at moderately reduced uricemia levels, as well as a non-reactive pattern. Discriminant analysis revealed 14 parameters of immunity, the constellation of which four clusters of metabolism of uric acid clearly distinguish. **Conclusion**. Endogenous uric acid exerts a modulatory immunotropic effect in healthy female rats.

***Key Words*:** uricosuria, uricemia, immunity, relationships, female rats.

INTRODUCTION

Previously, we found a wide range of uric acid metabolism parameters grouped into four clusters [11] and functional relationships of uricemia and uricosuria with the parameters of immunity in female rats analyzed [12]. It was found by canonical correlation analysis that the causal root receives a factor load from uricosuria twice that from uricemia. Judging by the factor loadings on the immune root, the most significant enhancing effect of endogenous uric acid is increase in the intensity and activity of the phagocytosis of microbes by neutrophils (but not monocytes) of blood. In addition, uric acid increases the relative content of lymphocytes in general and B-lymphocytes in particular in the blood and T-lymphocytes in the thymus. Less significant enhancing effect of uric acid on the increase in the content of fibroblasts in the spleen and macrophages in the thymus, as well as the increase in entropy of the immunocytogram of blood. On the other hand, uric acid significantly reduces the total blood content of leukocytes and the proportion of monocytes and young forms of neutrophils in leukocytogram as well as natural killer cells in immunocytogram. In addition, uric acid reduces the entropy of thymocytogram and the proportion of epitheliocytes and reticulocytes in it as well as of eosinophils in the splenocytogram. Taken together, both parameters of uric acid metabolism determine the immunity status of healthy female rats by 71% [12]. The purpose of this study is to determine the features of immune status in rats with quantitatively and qualitatively different uric acid metabolism.

MATERIAL AND METHODS

Experiment was performed on 60 healthy female Wistar rats 220-300 g. Among them 10 animals remained intact, using tap water from drinking ad libitum. The rats of others groups for 6 days administered through the tube various fluids (natural mineral waters and salt solution) at a dose of 1,5 mL/100 g of body mass.

The day after the completion of the drinking course in all rats, at first, a sample of peripheral blood (by incision of the tip of the tail) was taken for leukocytogram analysis. Animals were then placed in individual chambers with perforated bottom for collecting daily urine. The experiment was completed by decapitation of rats in order to collect as much blood as possible.

The serum and urine levels of the uric acid (uricase method) were determined. The analyzes were carried out according to the instructions described in the manual [9]. The analyzers “Pointe-180” ("Scientific", USA) were used with appropriate sets.

In the blood, the parameters of immunity were determined according to the tests of the 1st and 2nd levels of the WHO, as described in the manual [23]: the relative content of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep, their theophylline-resistant (T-helper) and theophylline-sensitive (T-cytolytic) subpopulations (by the test of sensitivity of rosette formation to theophylline); the population of B-lymphocytes (by the test of complementary rosette formation with erythrocytes of sheep) as well as blasttransformation of T-lymphocytes by PhHA. Natural killers were identified as large

granules contain lymphocytes.

About the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytic index, the microbial count and the killing index for Staphylococcus aureus (ATCC N25423 F49) [5,20,25].

After decapitation, the spleen and thymus were removed from the animals. Immune organs weighed and made smears-imprints for counting splenocytogram and thymocytogram [2,3]. For them, as well as immunocytogram and leukocytogram of blood, Shannon's entropy was calculated [13,24].

Digital material is statistically processed on a computer using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

Because analysis of variance revealed that the η^2 criterion for uricemia was significantly greater than that for uricosuria (0,95 versus 0,25) [11], uricemia was chosen as a main parameter in determining the characteristics of immune status in various uric acid metabolism states.

First of all, the relation between the normalized parameters (Z-scores) of uricemia and uricosuria is shown to be positive but not linear (Figs. 1 and 2). In particular, hypouricemia (cluster centroid: -1,18±0,05) is associated with hypo- and normouricosuria (centroid: -0,45±0,06). Uricemia of the lower zone of norm (centroid: -0,32±0,05) corresponds around the zero zone of uricosuria (centroid: -0,05±0,11). Instead, moderate (centroid: +0,59±0,06) and severe (centroid: +2,10±0,59) hyperuricemia are accompanied by a wide range of uricosuria.

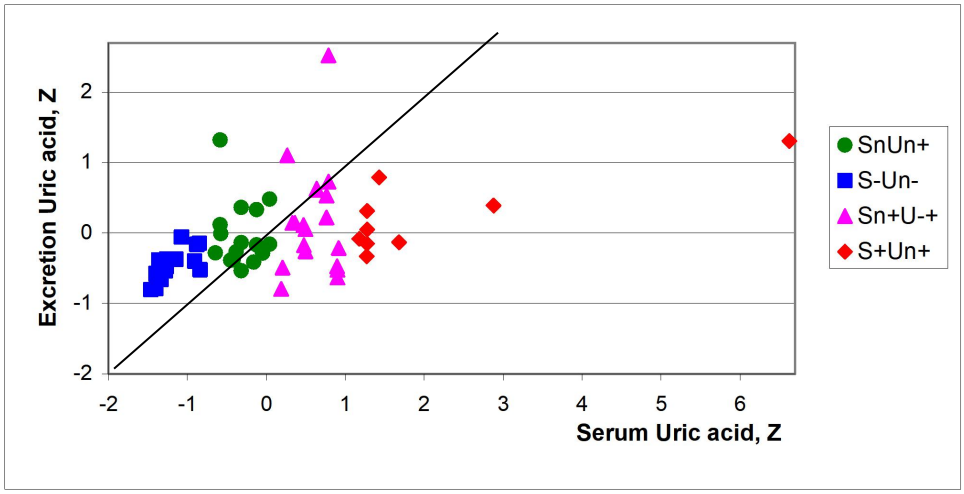

Fig. 1. Normalized levels (Z-scores) of uricemia (X-line) and uricosuria (Y-line) in female rats of different clusters

According to the results of the screening, three pairs of patterns of immune accompaniment of uricemia were formed.

The first pair of patterns displays quasilinear links, both immunoenhancing and immunosuppressing (Fig. 2). In particular (Table 2), hypouricemia is accompanied by decreased levels of T-lymphocytes in the thymus and their cytolytic subpopulation, as well as population of 0-lymphocytes in the blood, entropy of immunocytogram, fibroblasts in the spleen, and intensity of microbial phagocytosis by neutrophils. The two quasi-zero clusters of uricemia correspond to

the quasi-zero levels of this constellation of immunity parameters, whereas hyperuricemia causes them to increase. On this basis, the pattern is nominated immunoenhancing.

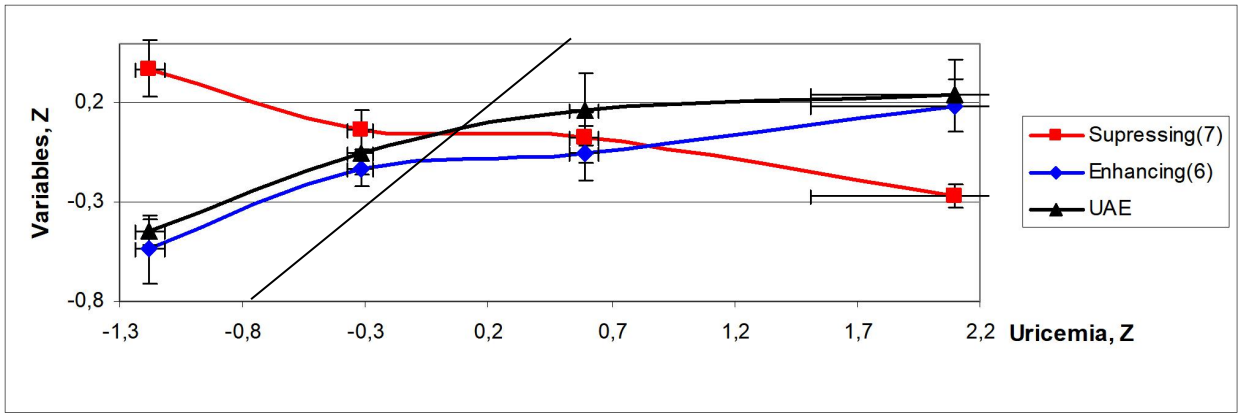

Fig. 2. The first pair of patterns of associations between uricemia (X-axis) and uricosuria and immunity parameters (Y-axis)

Instead, another immune constellation (thymus plasmocytes, thymocytogram entropy, spleen reticulocytes, stab neutrophils, natural killer cells and monocytes of blood and their microbial count) is suppressed in hyperuricemia, whereas in normouricemia it is in the normal range, and in case of hypouricemia it is activated. This reflects the immunosuppressing effect of uric acid on these parameters.

The second pair of patterns reflects the well-known U-shaped dependence (Fig. 3). In this case, immune extremes are observed in a cluster of moderately elevated uricemia. In particular, the upper boundary level is shown by macrophages and Hassal's lobules of the thymus, macrophages and entropy of the splenocytogram, T-helper cells and polymorphonuclear neutrophils of the blood. Instead, minimal levels are found for epitheliocytes of the thymus, neutrophils and lymphocytes of the spleen, basophils and B-lymphocytes of the blood, as well as for the activity and completion of microbial phagocytosis by blood neutrophils. Changes in the level of uricemia, both right and left, are accompanied by a decrease (U-minus-like pattern) or an increase (U-plus-like pattern) levels of immune parameters.

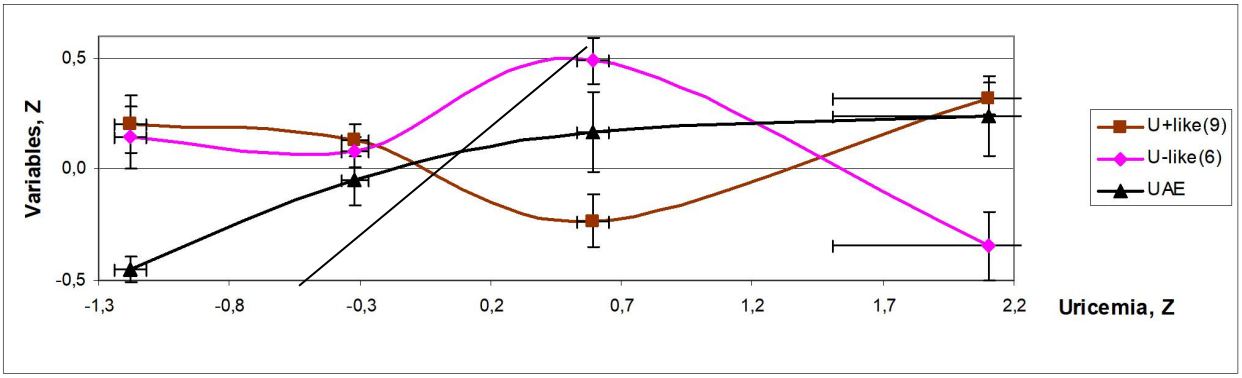

Fig. 3. The second pair of patterns of associations between uricemia (X-axis) and uricosuria and immunity parameters (Y-axis)

The third pair of patterns is characterized by the localization of optimal quasi-zero points of immune parameters in a cluster of moderately reduced uricemia. Differentially directed deviations of the uricemia level are accompanied by an increase in the level of reticulocytes in the thymus, lymphoblasts in the spleen, plasmocytes in the blood and entropy of the leukocytogram (U-enhancing pattern), on the one hand, and a decrease in the mass of the spleen and the content in the splenocytogram of plasmocytes, as well as lymphoblasts in the thymus and leukocytes in the blood (U-supressing pattern) - on the other hand (Fig. 4).

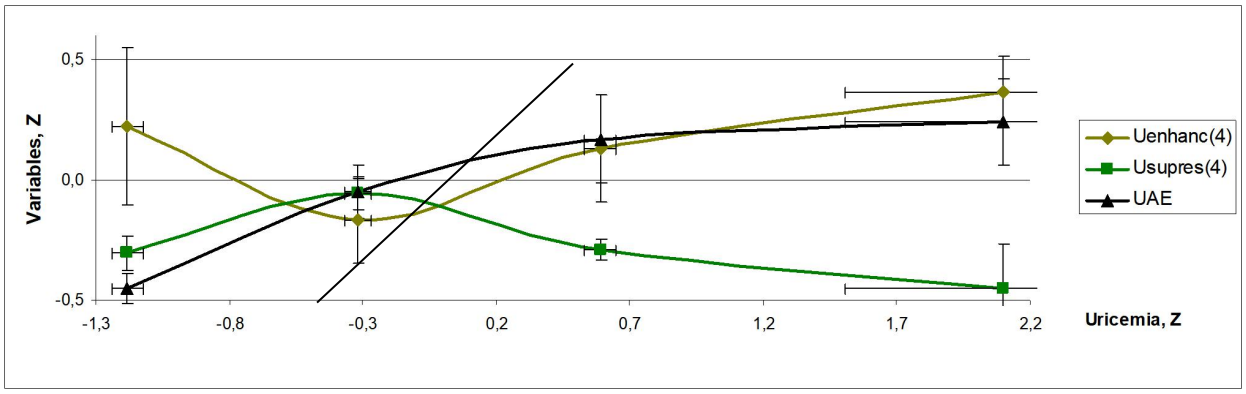

Fig. 4. The third pair of patterns of associations between uricemia (X-axis) and uricosuria and immunity parameters (Y-axis)

And only 7 of the 41 registered immune parameters (thymus mass, endothelial cell content in the thymocytogram, eosinophils in the spleen, eosinophils and common lymphocytes in the blood, as well as phagocytic index of monocytes and blasttransformation reaction of T lymphocytes) remain stably normal at qualitatively different levels of uricemia and uricosuria (areactive pattern) (Fig. 5).

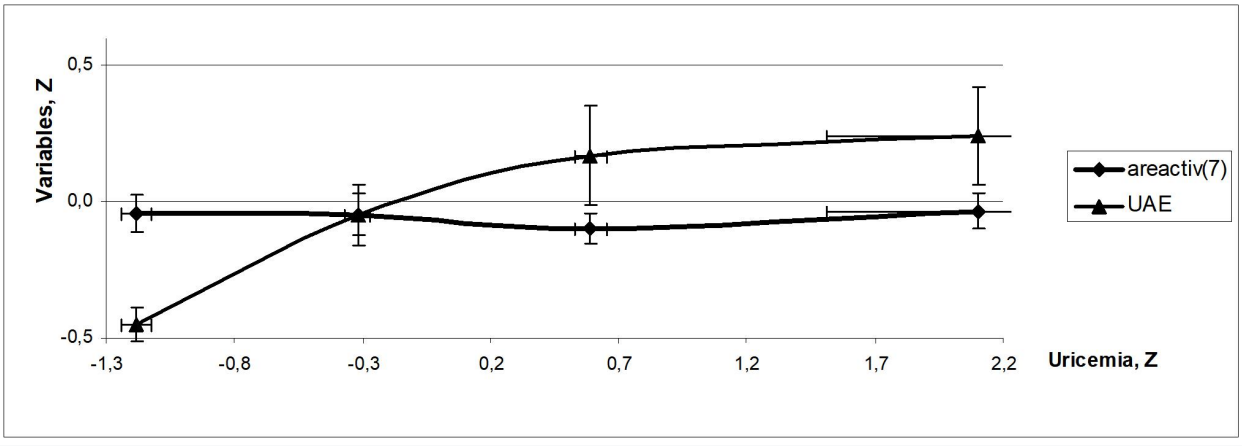

Fig. 5. Pattern of lack of response of parameters of immunity to the state of metabolism of uric acid

Discriminant analysis (method forward stepwise [19]) was conducted to identify exactly those immune parameters, in which the uric acid metabolism clusters differ significantly from each other. Only 15 variables were selected for inclusion in the model (3 from **thymus**, 4 from

spleen, 7 from **blood** as well as **uricemia**), while others, including uricosuria (?), were outside the discriminatory model (Tables 1 and 2).

Table 1. Summary of Stepwise Analysis for Immune Variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Uricemia, µM/L	46	10^{-6}	,290	45,6	10^{-6}
Macrophages Thymus, %	4,3	,008	,235	19,5	10^{-6}
0 Lymphocytes Blood, %	4,3	,008	,189	14,3	10^{-6}
Neutrophils Spleen, %	3,1	,036	,161	11,6	10^{-6}
T helper Lymphocytes, %	2,6	,061	,140	10,0	10^{-6}
Plasmocytes Thymus, %	2,5	,070	,122	8,9	10^{-6}
Entropy Splenocytogram	2,2	,101	,108	8,0	10^{-6}
Leukocytes Blood, G/L	2,1	,117	,096	7,4	10^{-6}
PMN Neutrophils, %	1,7	,185	,087	6,8	10^{-6}
Stab Neutrophils, %	1,4	,269	,080	6,3	10^{-6}
Spleen Mass Index, g/g BM	1,3	,289	,074	5,9	10^{-6}
Macrophages Spleen, %	1,7	,179	,066	5,6	10^{-6}
B Lymphocytes, %	1,4	,256	,060	5,3	10^{-6}
T cytolytic Lymphocytes, %	1,6	,209	,054	5,1	10^{-6}
Lymphoblastes Thymus, %	1,3	,282	,050	4,9	10^{-6}

Table 2. Discriminant Function Analysis Summary for Immune Variables ranked by Structural coefficient

Step 15, N of vars in model: 15; Grouping: 4 grps

Wilks' Lambda: ,0498; approx. $F_{(45)}$=4,9; $p<10^{-6}$

	Clusters of Uric acid Exchange (n)				Parameters of Wilks' Statistics					
Variables currently in the model	S+Un+ (9)	Sn+U± (19)	SnUn+ (17)	S-Un- (15)	Wil-ks' Λ	Parti-al Λ	F-re-move	p-level	Tole-rancy	Norm (10)
Uricemia, µM/L	1379	865	554	259	,215	,232	46,4	10^{-6}	,648	662
0 Lymphocytes, %	23,1	22,8	21,8	15,5	,063	,789	3,74	,018	,626	22,2
T cytolytic Lymph, %	16,4	16,0	15,9	15,8	,055	,900	1,56	,214	,504	16,0
Plasmocytes Thym, %	1,67	1,83	1,88	2,40	,061	,820	3,08	,037	,681	1,80
Stab Neutrophils, %	3,11	3,16	3,25	3,40	,052	,958	,62	,606	,482	3,60
Macrophages Thy, %	2,11	3,56	2,88	3,07	,063	,788	3,76	,016	,599	2,70
T helper Lymphoc, %	28,4	32,0	31,3	30,5	,062	,800	3,51	,023	,653	31,5
Macrophags Spleen,%	7,89	9,11	8,18	7,87	,055	,904	1,48	,234	,667	7,90
Entropy Splenocytogr	0,741	0,761	0,751	0,752	,063	,790	3,72	,018	,646	0,753
PMN Neutrophils, %	26,4	29,3	25,8	27,8	,055	,912	1,35	,272	,498	26,0
Neutrophils Spleen, %	13,9	11,8	13,1	13,8	,054	,918	1,26	,302	,793	13,0
B Lymphocytes, %	16,3	15,1	16,4	16,3	,056	,882	1,88	,148	,475	16,0
Spleen MInd, g/g BM	0,304	0,287	0,321	0,264	,059	,847	2,53	,070	,654	0,312
Lymphoblasts Thy, %	6,67	7,06	7,41	7,27	,054	,914	1,32	,282	,744	7,40
Leukocytes Blood,G/L	11,56	11,40	11,98	11,35	,059	,841	2,64	,062	,551	12,68
Variables currently not in the model	S+Un+ (9)	Sn+U± (19)	SnUn+ (17)	S-Un- (15)	Wil-ks' Λ	Parti-al Λ	F to enter	p-level	Tole-rancy	Norm (10)
Uricosuria, µM/100g•d	7,00	6,63	5,46	3,31	,048	,957	,61	,613	,738	5,72
Entropy Immunocyt	0,888	0,883	0,878	0,874	,048	,968	,45	,721	,716	0,874
Microb Count Neutro	8,2	8,0	7,8	7,3	,049	,992	,10	,957	,801	8,6
Lymphocyts Thym, %	70,7	69,2	69,5	68,2	,049	,993	,10	,962	,587	70,3
Fibroblasts Spleen, %	8,3	8,4	8,1	7,2	,049	,986	,19	,904	,707	8,2
Microb Count Monoc	4,1	4,7	4,6	5,0	,048	,964	,51	,676	,819	5,0
Reticulocyts Spleen,%	14,20	15,11	15,06	15,13	,049	,974	,36	,783	,648	14,30
Entropy Thymocytogr	0,531	0,556	0,551	0,570	,049	,986	,19	,902	,488	0,538
Monocytes Blood, %	3,67	4,11	5,12	5,87	,049	,981	,26	,854	,758	4,80
Natur Killers Blood,%	15,1	15,6	15,7	16,6	,049	,985	,21	,888	,741	15,6
Hassal corp Thym, %	1,56	2,06	1,88	2,07	,048	,963	,52	,672	,710	1,70
Killing Ind Neutro,%	52,1	51,3	53,7	55,1	,048	,966	,48	,700	,802	50,7
Phagoc Ind Neutro, %	70,9	68,8	69,7	69,4	,048	,972	,39	,762	,829	69,5
Lymphocyts Spleen,%	49,0	47,7	48,2	48,3	,049	,989	,16	,924	,150	48,7
Epitheliocyt Thym, %	9,6	8,9	9,2	9,6	,049	,990	,14	,933	,517	8,8
Basophiles Blood, %	0,44	0,26	0,35	0,27	,046	,932	1,00	,403	,711	0,30
Plasmocytes Blood, %	0,83	0,80	0,58	0,96	,048	,957	,62	,608	,446	0,47
Entropy Leukocytogr	0,616	0,577	0,558	0,565	,048	,973	,38	,769	,782	0,596
Lymphoblas Spleen,%	4,00	4,16	3,76	4,27	,050	,999	,01	,998	,405	3,90
Reticulocyts Thym, %	5,11	4,83	4,47	4,80	,049	,977	,32	,814	,665	4,70
Plasmocyts Spleen, %	1,44	2,05	2,18	1,93	,049	,980	,28	,836	,457	2,50
Thymus Mass Index	0,028	0,026	0,030	0,032	,049	,978	,30	,824	,575	0,028
Endotheliocyts Thy,%	2,67	2,56	2,76	2,60	,047	,941	,86	,472	,499	2,60
RBTL on PhHA, %	76,8	75,9	78,3	78,4	,047	,942	,84	,478	,705	78,8
Eosinophils Spleen, %	1,22	1,63	1,41	1,53	,049	,980	,28	,839	,707	1,50
Eosinophiles Blood, %	3,89	3,79	3,47	3,67	,049	,986	,20	,897	,848	4,60
Phagoc Ind Monoc, %	3,00	2,95	2,74	2,83	,048	,973	,37	,772	,711	2,90
Lymphocyts Blood, %	62,4	59,3	62,1	59,0	,050	,998	,03	,992	,133	60,7

The distinctive information contained in the 15 discriminant variables is condensed into three roots. The first root contains 75,5% of the discriminatory potential (r^*=0,918; Wilks' Λ=0,050; $\chi^2_{(45)}$=148; $p<10^{-6}$), second root contains 20,3% (r^*=0,768; Wilks' Λ=0,316; $\chi^2_{(28)}$=57; $p<10^{-3}$), and the third root is even smaller and no significant: 4,2% (r^*=0,478; Wilks' Λ=0,772; $\chi^2_{(13)}$=13; p=0,467).

Calculating the values of the discriminant root for each animal as the sum of the product of the raw coefficients on the individual values of the discriminant variables together with a constant (Table 3) makes it possible to visualize each rat in the information space of the roots (Figs. 5 and 6).

Table 3. Standardized and Raw Coefficients and Constants for Canonical Variables

Coefficients	Standardized			Raw		
Variables	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Uricemia, µM/L	-1,183	-,008	-,153	-,0049	-,00003	-,0006
Macrophages Thymus, %	,205	,732	,088	,194	,694	,084
0 Lymphocytes Blood, %	-,558	,238	,423	-,081	,035	,061
Neutrophils Spleen, %	,206	-,326	-,149	,108	-,171	-,078
T helper Lymphocytes, %	,181	,682	,140	,050	,189	,039
Plasmocytes Thymus, %	,475	-,312	-,278	,593	-,390	-,348
Entropy Splenocytogram	-,387	,571	-,164	-17,64	26,01	-7,489
Leukocytes Blood, G/L	-,460	,350	,405	-,098	,075	,087
PMN Neutrophils, %	,050	,520	-,252	,008	,081	-,039
Stab Neutrophils, %	,095	-,360	-,132	,082	-,309	-,114
Spleen Mass Index, g/g BM	-,296	-,235	,747	-4,586	-3,636	11,57
Macrophages Spleen, %	,192	,403	-,269	,110	,231	-,154
B Lymphocytes, %	,047	-,631	,229	,015	-,200	,073
T cytolytic Lymphocytes, %	-,373	,347	-,213	-,117	,109	-,067
Lymphoblastes Thymus, %	,002	,303	,519	,0023	,308	,528
		Constants		16,39	-28,94	-,470
		Eigenvalues		5,350	1,440	,296
	Cumulative Properties			,755	,958	1,000

As we can see, on the plane of the first two roots, in which 95,8% of the information is condensed, the distinction between clusters is quite clear. The localization of the members of the **S-Un-** cluster in the right (positive) zone of the axis of the first root reflects (Table 4) the combination of hypouricemia with a increased content in the thymus of plasmocytes and a reduced content in the blood of 0-Lymphocytes at normal levels of T-cytolytic lymphocytes and stab neutrophils.

The opposite left (negative) region of the axis is occupied by the members of the **S+Un+** cluster, which reflects the combination of hyperuricemia with a decreased content of plasmocytes in the thymus and stab neutrophils in the blood and an increased content of Tc and 0 lymphocytes. The central (around zero) zone of the axis is occupied by members of the other two clusters, in which the normal level of uricemia is accompanied by the normal levels of the mentioned immune parameters.

It seems that uric acid upregulates the content of T-cytolytic and 0-Lymphocytes in the blood while downregulates the content of stab neutrophils in the blood and plasmocytes in the thymus.

It is noteworthy that the members of the clusters **Sn+U-+** and **SnUn+** are not clearly distinguished along the axis of the first root (distance between centroids is 1,46). The latter is much larger along the axis of the second root (1,84), due to the top position of the cluster **Sn+U-+**. This position reflects elevated levels in its members thymus and spleen macrophages and blood polymorphonuclear neutrophils, as well as entropy of the splenocytogram in combination with reduced levels of spleen neutrophils and blood B-lymphocytes. However, in this cluster, the level of T-helper cells is normal, while in others clusters it is reduced.

It seems that the upper boundary level of uricemia upregulates/downregulates the levels of 6 immune parameters, without affecting the level of T-helper cells, and both the decrease and increase of it is accompanied by a decrease in the levels of these immune parameters to normal, and T-helper - even lower (Table 4).

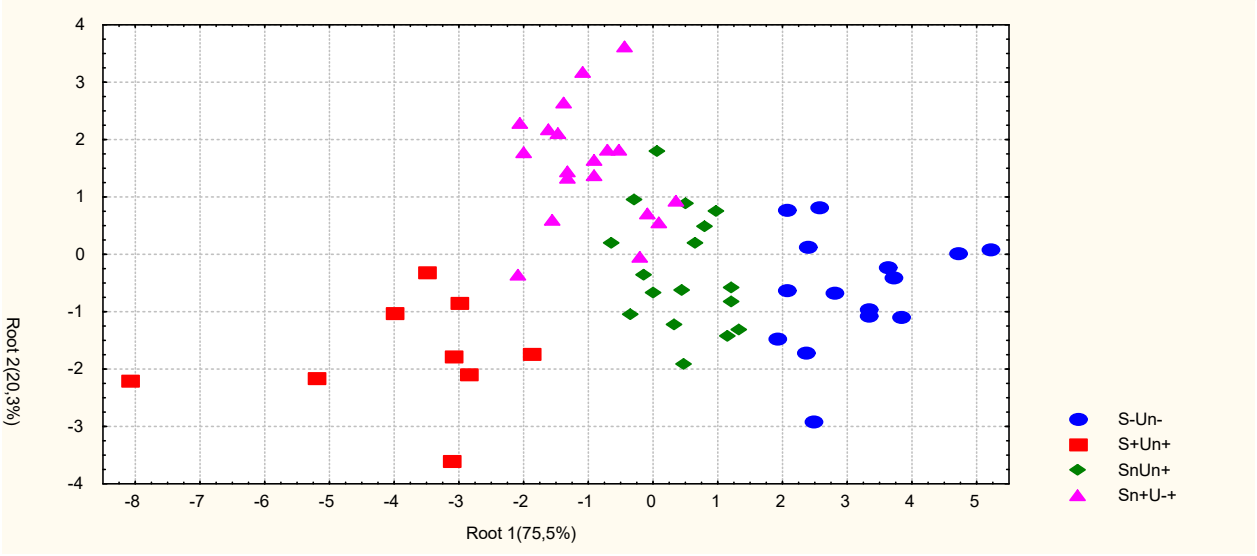

Fig. 6. Scatterplot of rats of differ clusters in space of first and second Roots

Table 4. Correlations Variables-Canonical Roots, Means of Roots and Z-scores of Variables

	Correlations Variables-Roots			S+Un+	Sn+U-+	SnUn+	S-Un-
Root 1(75,5%)	Root 1	Root 2	Root 3	**-3,86**	**-1,01**	**+0,45**	**+3,09**
Uricemia	**-,669**	-,053	-,402	**+2,10**	**+0,59**	**-0,32**	**-1,18**
Uricosuria	currently not in the model			**+0,24**	**+0,17**	**-0,05**	**-0,45**
0 Lymphocytes Blood	**-,172**	,114	,324	**+0,15**	**-0,10**	**-0,07**	**-1,08**
T cytolytic Lymphocytes	**-,026**	-,024	-,026	**+0,19**	**-0,02**	**-0,05**	**-0,08**
Plasmocytes Thymus	**,135**	-,041	-,215	**-0,17**	**+0,04**	**+0,10**	**+0,76**
Stab Neutrophils	**,038**	-,020	-,026	**-0,45**	**-0,41**	**-0,34**	**-0,19**
Root 2(20,3%)				-1,75	**+1,56**	-0,28	-0,62
Macrophages Thymus	,072	**,347**	-,079	-0,44	**+0,64**	+0,16	+0,27
T helper Lymphocytes	,047	**,245**	,195	-0,99	**+0,14**	-0,07	-0,31
Macrophages Spleen	-,042	**,248**	-,015	-0,01	**+0,76**	+0,17	-0,02
Entropy Splenocytogram	,035	**,241**	-,040	-0,43	**+0,25**	-0,08	-0,03
PMN Neutrophils	,002	**,143**	-,280	+0,07	**+0,48**	-0,03	+0,27
Neutrophils Spleen	,051	**-,366**	-,076	+0,63	**-0,82**	+0,08	+0,56
B Lymphocytes Blood	,023	**-,147**	,107	+0,11	**-0,32**	+0,12	+0,09
Root 3(4,2%)				-0,34	-0,20	**+0,82**	-0,47
Spleen Mass Index	-,071	-,041	**,544**	-0,08	-0,25	**+0,09**	-0,48
Lymphoblastes Thymus	,088	,033	**,285**	-0,87	-0,41	**+0,01**	-0,16
Leukocytes Blood	-,002	-,012	**,102**	-0,19	-0,21	**-0,12**	-0,22

Along the axis of the third root (Fig. 7), members of the **SnUn+** cluster occupy the top position. This reflects their normal: the mass of the spleen, the level of lymphoblasts in the thymus and leukocytes in the blood, whereas in the members of other clusters these parameters are reduced (Table 4). Therefore, the lower boundary level of uricemia is optimal for these immune parameters, whereas its deviation in either direction causes their decrease.

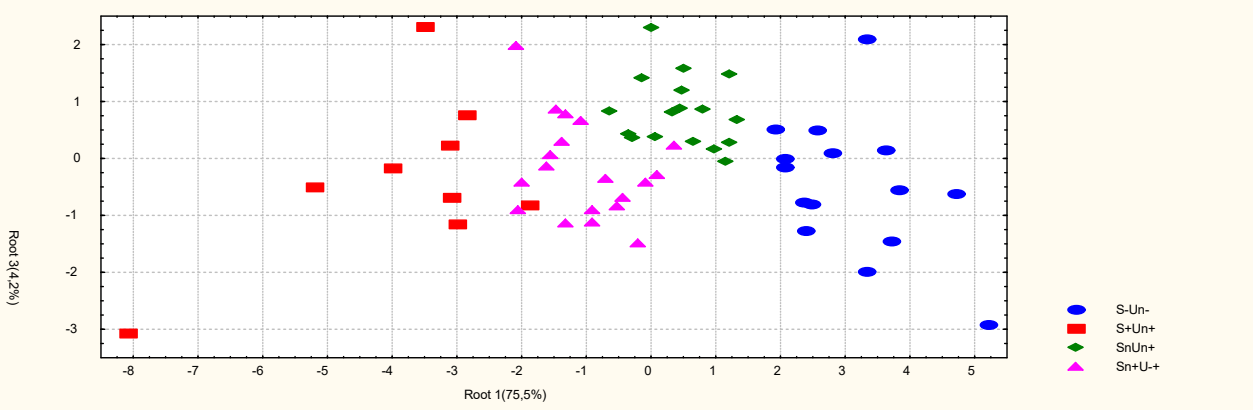

Fig. 7. Scatterplot of rats of differ clusters in space of first and third Roots

In general, in the information space of the three discriminant roots, all four clusters are clearly separated from each other, that is, they differ from each other in terms of uricemia and constellation of the 14 parameters of immunity. This distinction is documented by the calculation of the Mahalanobis distances between clusters (Table 5).

Table 5. Squared Mahalanobis Distances between clusters (above the diagonal), F-values (df=15,4) and p-levels (under the diagonal)

Clusters	S-Un-	S+Un+	SnUn+	Sn+U-+
S-Un-	0	53	9	23
S+Un+	13,5 10^{-6}	0	24	20
SnUn+	3,5 10^{-3}	6,3 10^{-6}	0	7
Sn+U-+	9,1 10^{-6}	5,6 10^{-5}	3,0 0,003	0

The use of classification functions (Table 6) makes it possible to retrospectively identify two clusters without error and the other two with two errors (Table 7), ie the overall recognition accuracy is 93,3%.

Table 6. Coefficients and Constants of Classification Functions for Immune accompaniment of Uric acid metabolism clusters

Clusters	S+Un+	Sn+U-+	SnUn+	S-Un-
Variables	p=,150	p=,317	p=,283	p=,250
Uricemia, µM/L	,107	,093	,085	,073
Macrophages Thymus, %	19,85	22,70	21,80	21,97
0 Lymphocytes Blood, %	3,544	3,436	3,317	3,012
Neutrophils Spleen, %	3,524	3,254	3,647	4,092
T helper Lymphocytes, %	2,244	3,017	2,784	2,802
Plasmocytes Thymus, %	-20,67	-20,32	-19,09	-16,95
Entropy Splenocytogram	2603	2638	2557	2511
Leukocytes Blood, G/L	6,829	6,809	6,616	6,219
PMN Neutrophils, %	3,168	3,453	3,276	3,320
Stab Neutrophils, %	-8,333	-9,136	-8,566	-8,099
Spleen Mass Index, g/g BM	81,66	58,24	69,98	44,18
Macrophages Spleen, %	-,430	,624	,206	,616
B Lymphocytes, %	-5,624	-6,232	-5,770	-5,756
T cytolytic Lymphocytes, %	11,09	11,11	10,67	10,41
Lymphoblastes Thymus, %	14,46	15,56	15,53	14,75
Constants	-1314	-1355	-1277	-1228

Table 7. Classification Matrix for clusters of Uric acid metabolism
Rows: Observed classifications; Columns: Predicted classifications

Clusters	Percent Correct	S+Un+ p=,150	Sn+U-+ p=,317	SnUn+ p=,283	S-Un- p=,250
S+Un+	100	9	0	0	0
Sn+U-+	89,5	0	17	2	0
SnUn+	88,2	0	2	15	0
S-Un-	100	0	0	0	15
Total	93,3	9	19	17	15

In conclusion, we consider it appropriate to re-brand patterns created only from discriminant immune variables (Fig. 8).

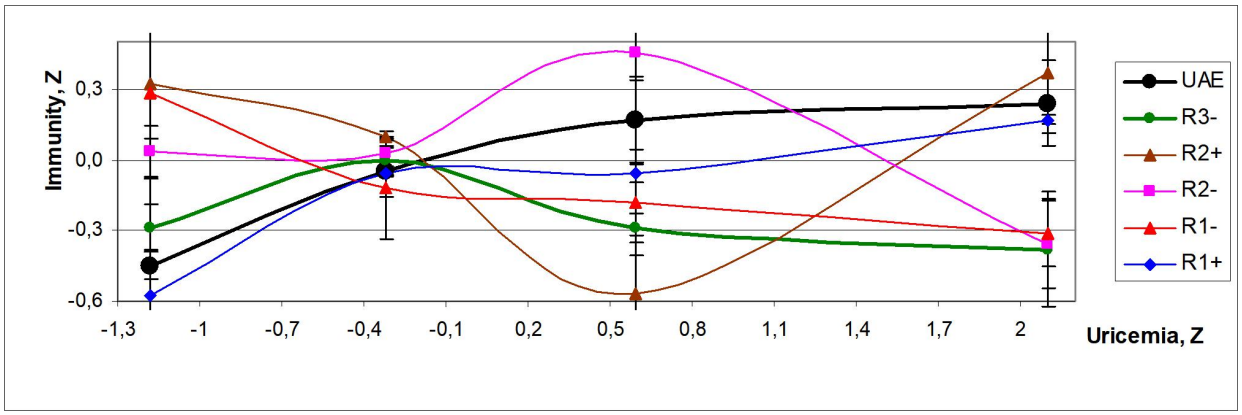

Fig. 8. Variants of relationships between Uricemia (X-line) and Uricosuria as well as Immune variables (Y-line) condensed in discriminant Roots (R)

As you can see, the reduction in the number of variables did not significantly affect the image of the patterns, but did not become a U-enhancing pattern.

Therefore, 34 immune parameters from the 41 reported in this study were in one way or another related to uric acid metabolism, mainly uricemia. This is consistent with the concept of the physiological and pathophysiological role of uric acid, based on the structural homology of its molecule with adenosine and methylxanthines molecules [4,6-8,14,15,18,21,26,28].

The immunotropic effect of adenosine is realized through its receptors (A_1, A_{2A}, A_{2B}, A_3), which express virtually all populations of immunocytes: T, NK, B lymphocytes, macrophages, neutrophils, dendritic and endothelial cells [1,16,17,27].

A non-selective adenosine receptor antagonisti, mainly A_{2A}, are caffeine and other methylxanthines [21,22] which are introduced into the human body almost daily from coffee, tea and cocoa. We hypothesize that uric acid is an **endogenous** non-selective adenosine receptor antagonist. We hope that the results presented in this article have made a modest contribution to support this hypothesis.

CONFORMITY TO ETHICAL STANDARDS

Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research.

The carrying out of experiments was approved by the Ethics Committee of the I. Horbachevskyi Ternopil' National Medical University. The modern rules for the maintenance and use of laboratory animals complying with the principles of the European Convention for the Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).

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